ABSTRACT N-(4-Hydroxyphenyl)-retinamide (4-HPR; Fenretinide) is a synthetic retinoid which is undergoing investigation as a cancer chemopreventive agent. However, 4-HPR alters vitamin A kinetics and reduces the concentration of plasma retinol. We have conducted studies to examine the effects of 4-HPR on the activity of the enzyme lecithin:retinol acyltransferase (LRAT). This enzyme is implicated in the absorption and storage of vitamin A and is regulated, in liver, by vitamin A nutritional status. To determine whether 4-HPR, like retinoic acid, is able to induce liver LRAT activity, vitamin A-deficient rats having negligible liver LRAT activity were treated with single doses of 4-HPR (0.02–2.5 mg) and liver homogenates were assayed for LRAT activity using 3H-retinol bound to the cellular-retinol binding protein, CRBP, as substrate. Treatment with 4-HPR resulted in a dose- and time-dependent increase in liver LRAT activity which reached a maximum at 24 h. The activity of LRAT assayed in vitro and of hepatic 3H-retinyl ester content determined after an in vivo pulse of 3H-retinol were highly correlated (r = 0.802, P < 0.0002). When vitamin A-sufficient rats were fed a 4-HPR-supplemented diet for 30 d, LRAT activity differed significantly from control values in the liver (P < 0.0001) but not the small intestines. Changes in hepatic retinol metabolism which favor the esterification of vitamin A may be related to the mechanism by which 4-HPR alters vitamin A kinetics in vivo.

INDEXING KEY WORDS:
- retinoic acid
- cellular retinol-binding protein
- retinol esterification
- retinamide
- rats
- vitamin A

N-(4-Hydroxyphenyl)-retinamide (4-HPR; Fenretinide), an amide analog of vitamin A acid (retinoic acid), was first synthesized by Moon et al. (1979) who showed its ability to inhibit the formation of mammary gland tumors in rats and to reverse the keratinization phenotype of organ cultures from vitamin A–deficient hamster trachea. Subsequently, both animal and human studies have demonstrated that 4-HPR has the potential to inhibit the development of a variety of cancers of epithelial origin (see Chiesa et al. 1992, Hill and Grubbs 1992). The potential utility of 4-HPR is further enhanced by its low toxicity compared with retinoic acid and a number of other synthetic retinoids (Costa et al. 1989, Moon et al. 1979).

A question of basic importance for the long-term therapeutic use of 4-HPR, or similar retinoids, is whether it influences the normal metabolism of vitamin A. In the case of 4-HPR, Formelli et al. (1987 and 1989) reported that the concentrations of both plasma retinol and retinol-binding protein (RBP) declined rapidly after the administration of 4-HPR. The underlying mechanism for this change in retinol dynamics has been partly elucidated. In vivo kinetic studies of 4-HPR–treated rats have shown a reduction in the residence time for retinol in plasma (Lewis et al. 1994) and a decreased rate of plasma retinol turnover (Adams et al. 1995). It is known that 4-HPR binds in the retinol-binding cavity of RBP, interfering with the association of RBP with its co-transport protein transthyretin (Berni et al. 1993). More RBP is present in the extravascular space after treatment with 4-HPR (Ritter et al. 1995).
Hepatic retinol metabolism is central to the whole-body economy of vitamin A, and the effects of 4-HPR on retinol metabolism in liver have not been studied in detail. In the experiments presented here, we have examined whether 4-HPR, similar to retinoic acid (Matsuura and Ross 1993), is able to regulate the activity of the microsomal enzyme lecithin:retinol acyltransferase (LRAT) which is active in liver, small intestine, the retina and possibly other tissues, and has been implicated in the intestinal formation of chylomicron retinyl esters and in retinyl ester storage in the liver and retina (see Ross 1993a for review). As a substrate, LRAT is capable of utilizing retinol bound to one of the cellular retinol-binding proteins (CRBP in liver and CRBP-II in the intestine) and a fatty acid from the sn-1 position of phosphatidylcholine, forming mainly retinyl palmitate and stearate. Randolph and Ross (1991) have shown that the activity of liver LRAT is highly sensitive to vitamin A nutritional status. During the course of vitamin A depletion in rats, hepatic LRAT activity (assayed with a saturating concentration of CRBP-bound retinol) decreased progressively until it reached undetectable levels as rats became vitamin A deficient. This effect was specific for liver, because no significant decrease in activity was observed for intestinal LRAT or for another liver enzyme, and specific for LRAT because no change was observed in the activity of acyl-CoA:retinol acyltransferase (ARAT, which esterifies retinol but does not utilize retinol bound to CRBP). The activity of hepatic LRAT was restored rapidly after vitamin A–deficient rats were repleted with a single oral dose of retinol (Randolph and Ross 1991) or after treatment with retinoic acid in the absence of retinol (Matsuura and Ross 1993). The restoration of hepatic LRAT activity by retinoic acid was shown to be dose- and time-dependent and was blocked completely in vivo by inhibitors of RNA and protein synthesis (Matsuura and Ross 1993). Based on these results, it has been proposed that low LRAT activity during vitamin A deficiency serves to limit the esterification of retinol, thus sparing retinol for oxidation to retinoic acid or recycling to plasma and distribution to peripheral tissues (Randolph and Ross 1991, Ross 1993b). Conversely, in the presence of adequate vitamin A and/or retinoic acid, LRAT activity is maintained so that most of the retinol entering the liver is directed into storage in the form of retinyl esters.

In the present study, we have investigated the effects of acute or chronic treatments with 4-HPR on LRAT activity in rat liver. Experiments were first conducted in vitamin A–deficient rats to determine whether 4-HPR has retinoic acid–like activity in inducing liver LRAT activity. In these experiments, we also examined the relationship between liver LRAT activity assayed in vitro and the rate of retinol esterification determined in vivo. A study was also conducted in normal, vitamin A–sufficient rats to determine whether the chronic dietary administration of 4-HPR, as used in models of cancer chemoprevention, affects the activity of LRAT in either the liver or the small intestines.

MATERIALS AND METHODS

Materials. The 4-HPR used in these studies was kindly provided by Dr. Richard C. Moon (University of Illinois at Chicago) and the R. W. Johnson Pharmaceutical Research Institute (Springhouse, PA). Retinoic acid, Tween 80 and all standard chemicals were purchased from Sigma Chemical (St. Louis, MO). Actinomycin D was purchased from Calbiochem (San Diego, CA), and tritiated retinol [1(N)-3H-vitamin A] from Dupont NEN Research Products (Boston, MA).

Animals and diets. Procedures were approved by the Institutional Animal Use and Care Committee of the Medical College of Pennsylvania. Male and female Lewis rats (Charles River Breeding Laboratories, Wilmington, DE) were given free access to drinking water and a nutritionally adequate semipurified diet (Bowman et al. 1990) containing 23 weight% protein (casein plus L-methionine), 56% digestible carbohydrates, and 10.5% fat (cottonseed oil). The control diet contained 4 mg of retinol (as retinyl palmitate) per kg diet (control diet), whereas the vitamin A–deficient diet was identical except for the lack of vitamin A. The vitamin A–deficient diet was fed to the lactating mothers of the rats used in these studies beginning on about d 5 of lactation. After weaning (d 20–21), pups were fed either the vitamin A–deficient or the control diet. At the time of administration of 4-HPR, all rats fed the vitamin A–deficient diet were vitamin A deficient by the criterion of a plasma retinol <0.2 µmol/L (male and female rats were 45 and 55 d old, respectively). The 4-HPR–supplemented diet contained 782 mg (2 mmol) 4-HPR/kg diet (Zhao et al. 1994) which was blended into the control diet, above, as described by Moon et al. (1979). Both male and female rats were used in these studies with similar results, but only one gender was used in a given experiment. Rats were allowed to consume feed ad libitum throughout the experiments.

Preparation and delivery of retinoids. Specific conditions for individual experiments are described in the text or legends to figures and tables. To prepare 4-HPR for intragastric or intraperitoneal repletion of vitamin A–deficient rats, 100 mg of 4-HPR was mixed with 100 µL of ethanol and 10 µL of Tween 80. The dissolved mixture was diluted to 5 mL with sterile PBS and filtered through a 0.45-µm filter. The concentration of 4-HPR in the filtered solution was determined by spectrophotometry at 362 nm [extinction coefficient 47,900 mol⁻¹·L⁻¹·cm⁻¹ (Moon et al. 1979)]. The solution was delivered either orally through a gastric feeding needle directly into the stomach or by intraperitoneal injection. For comparison with previous experiments, retinoic acid was prepared as described previously and de-
livered by the intraperitoneal route [Matsuura and Ross 1993]. The vehicle control contained all materials except retinoid.

To determine retinol esterification in vivo, we administered 100 µg of tritium-labeled retinol to rats 2 h before the end of the experiment (18 h after treatment with vehicle, 4-HPR or retinoic acid). Tritium-labeled retinol was mixed with unlabeled retinol (≈37 kBq/100 µg), purified on a column of aluminum oxide (Ross 1982), and stored in ethanol under nitrogen at −20°C. Just prior to intraperitoneal injection, the ethanol was removed by argon evaporation and the 3H-retinol was mixed with 100 µL of ethanol plus 10 µL of Tween 80. This mixture was diluted with sterile PBS to a retinol concentration of 100 g retinol/200 L and was administered by intraperitoneal injection, as above. Experiments with actinomycin D were performed as described previously [Matsuura and Ross 1993]. To determine the relationship between hepatic LRAT activity measured in vitro and the esterification of retinol in vivo, vitamin A–deficient rats were treated at time zero with either 20 µg of retinoic acid intraperitoneally or 0.5 mg of 4-HPR intragastrically, followed after 18 h by an intraperitoneal injection of 100 µg of 3H-retinol. Two hours later, livers were collected for determination of hepatic LRAT activity in vitro and of 3H-retinyl ester that had been synthesized in vivo.

In a preliminary study, we examined the effect of a single 20-mg dose of 4-HPR [≈80 mg 4-HPR/kg of body weight as used previously by Formelli et al. [1987] to study the effect of 4-HPR on plasma retinol] on the liver LRAT activity of vitamin A–sufficient rats. To study the effect of chronic dietary 4-HPR on liver and intestinal LRAT activity, twelve 63-d-old vitamin A–sufficient rats were divided into two groups of equal weight and were fed either the 4-HPR–supplemented diet or vehicle-containing control diet, above, for 30 d. Data on plasma and liver retinol concentrations for these rats have been reported previously [Zhao et al. 1994].

At the end of each experiment, rats were killed by carbon dioxide inhalation, blood was collected from the vena cava into heparinized syringes, and livers were removed and frozen rapidly in liquid nitrogen. Liver and plasma samples were stored at −70°C before analysis. In some experiments, the small intestines, separated into duodenum plus jejunum and ileum, were also collected, rinsed inside and outside with saline, and frozen as above.

**Liver homogenate preparation and LRAT assay.** Livers were thawed and homogenized at 4°C in a Potter–Elvehjem homogenizer using 4 mL of ice-cold homogenization buffer (0.28 mmol/L sucrose, 0.01 mmol/L potassium phosphate buffer, pH 7.25, with 1 mmol/L dithiothreitol) per gram of liver [Matsuura and Ross 1993]. In one series of experiments, washed liver microsomes were prepared as described previously [Yost et al. 1988]. The specific activity of liver LRAT was assayed with 5 µmol/L of 3H-retinol-CRBP [Yost et al. 1988] as substrate in a total volume of 0.15 mL containing 0.15 mmol/L K2HPO4 buffer, pH 7.4, and 2 mmol/L dithiothreitol at 37°C. Whole homogenates were assayed using protein concentrations and incubation times previously shown to be in the linear range for retinol esterification [Matsuura and Ross 1993, Randolph and Ross 1991]. Each sample of liver homogenate (1 mg protein) or microsomes (0.1 mg protein) was incubated in duplicate with 3H-retinol-CRBP, a boiled sample of the same preparation served as the background which was subtracted from all incubations. The esterified 3H-retinol product was separated from 3H-retinol by alumina column chromatography and radioactivity was determined by liquid scintillation counting [Randolph and Ross 1991]; the mean value of the duplicate incubations is reported. LRAT activity in homogenates of whole small intestine (separated into duodenum plus jejunum and ileum) was assayed similarly except that 1 mg of homogenate protein was incubated in a total volume of 0.15 mL with 1 µmol/L of 3H-retinol-CRBP.

**Statistics.** Results are presented as the mean ± SD for each treatment group. Statistical comparisons were made between treatment and control groups by an unpaired Student’s t test or by one-way ANOVA followed by a protected least significant difference test (Instat 1.11, Graph Pad, San Diego, CA). A P value = 0.05 was considered significant.

**RESULTS**

**Effect of acute administration of 4-HPR in vitamin A–deficient rats.** When 1.25 mg of 4-HPR was administered either intragastrically or intraperitoneally to vitamin A–deficient rats, the specific activity of LRAT in liver homogenates increased after 20 h from 0.2 (vitamin A–deficient) to ~8 pmol retinyl ester/(min · mg protein) [Table 1]. The in vitro addition of 4-HPR to liver microsomes prepared from vitamin A–deficient rats did not affect LRAT activity (Table 1).

The dose-response to intragastrically administered 4-HPR (0.02–2.5 mg) was examined in vitamin A–deficient rats. As is shown in Table 2, LRAT activity was markedly greater in rats administered doses of 0.1–2.5 mg of 4-HPR (P < 0.002).

Following treatment of vitamin A–deficient rats with a single intragastric dose of 0.5 mg of 4-HPR, he-
TABLE 1

Effect of 4-hydroxyphenyl retinamide (4-HPR) on lecithin:retinol acyltransferase (LRAT) activity in liver homogenates from vitamin A–deficient rats treated in vivo, or vitamin A–deficient rat liver microsomes treated in vitro

<table>
<thead>
<tr>
<th>4-HPR Dose</th>
<th>Hepatic LRAT activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>— mg/rat ——</td>
<td>pmol retinyl ester/(min·mg homogenate protein)</td>
</tr>
<tr>
<td>In vivo²</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>1.25 (oral)</td>
<td>7.7 ± 0.5*</td>
</tr>
<tr>
<td>1.25 (intraperitoneal)</td>
<td>7.8 ± 0.6*</td>
</tr>
<tr>
<td>In vitro³</td>
<td>1.0 ± 0.6</td>
</tr>
<tr>
<td>20 – µmol/L incubation ——</td>
<td>pmol retinyl ester/(min·mg homogenate protein)</td>
</tr>
<tr>
<td>0</td>
<td>0.9 ± 0.4</td>
</tr>
</tbody>
</table>

¹ Values are means ± SD. The n for in vivo experiments is the number of rats (female) and for in vitro assays the number of assay replicates.
² LRAT activities were measured 20 h after treatment in vivo.
³ Pools of liver microsomes prepared from livers of three vitamin A–deficient male rats were incubated for 4 min at 37°C with 20 µmol/L of 4-HPR or vehicle before assay of LRAT activity. Results shown are for one of three similar incubation experiments. The LRAT specific activities for microsomes are about 5- to 7-fold greater than those for comparable homogenates because washed microsomal protein comprise about 12–20% of the homogenate protein.

Correlation of LRAT activity assayed in vitro and hepatic retinol ester formation in vivo. As is shown in Figure 3 for 15 individual rat livers, there was a significant, overall linear relationship (r = 0.802, P < 0.0002) between the activity of LRAT assayed in liver homogenates in vitro and the esterification of 3H-reti-

4-HPR, mg 0 1 1
Act. D, mg/100 g 0 0.3

In vivo treatments

FIGURE 1 The kinetics of liver lecithin:retinol acyltransferase (LRAT) activity determined in vitamin A–deficient rats at various times after the administration of 0.5 mg of 4-hydroxyphenyl retinamide (4-HPR) intragastrically. Each data point represents an individual male rat.
Effect of acute or chronic 4-HPR administration on hepatic LRAT activity in vitamin A–sufficient rats. In a preliminary study using a single 20-mg intragastric dose of 4-HPR, liver LRAT activity was significantly greater in four 4-HPR-treated vs. four vehicle-treated male rats [20.0 ± 1.4 vs. 14.7 ± 1.3 pmol retinyl ester/ (min •mg homogenate protein), P < 0.03]. The addition of 4-HPR in vitro to control rat liver microsomes, as described above for microsomes of vitamin A–deficient rats, resulted in a relatively small (<8%) but statistically significant increase in LRAT activity [102 ± 2 vs. 94 ± 2 pmol retinyl ester/ (min •mg homogenate protein), P < 0.02].

In the main experiment with vitamin A–sufficient rats, we examined the effect of 4-HPR administered in the diet of female rats for 30 d. Table 3 shows that, as expected, the plasma retinol concentration of liver vitamin A (total retinol) did not differ significantly between the two groups.

Effect of acute or chronic 4-HPR administration on intestinal LRAT activity. In some of the experiments described above, LRAT activity was also measured in homogenates of small intestine. Twenty hours following administration of a single dose of 0.1 or 2.5 mg 4-HPR to vitamin A–deficient rats, intestinal LRAT activity was unchanged (data not shown). Following 30 d of dietary administration of 4-HPR to vitamin A–sufficient rats [Table 3] intestinal LRAT activity did not differ significantly in the duodenum plus jejunum, but was ~50% lower in the ileum (P < 0.002) compared with controls.

**DISCUSSION**

The chemopreventive properties of 4-HPR have been demonstrated in animal models of breast, bladder and prostate cancer, and in the prevention of human leukoplakia (Chiesa et al. 1992). Currently, 4-HPR is being tested in clinical studies of breast cancer chemoprevention (Cobleigh 1994, Decensi et al. 1993). Yet, rather little is known of the metabolism of this retinoid, its mechanism of action, or its effects on the metabolism of vitamin A. The concept that 4-HPR itself has vitamin A biological activity was first suggested by Moon et al. (1979) based on the ability of 4-HPR to promote the growth of vitamin A–deficient hamsters and induce epithelial differentiation in tracheal organ cultures. The potency of 4-HPR in hamsters and rats was about one tenth that of retinoic acid (Moon et al. 1979, Zhao et al. 1994). Although 4-HPR is far less toxic than either retinyl acetate or retinoic acid (Moon et al. 1979), it causes a rapid and sustained reduction of plasma retinol and RBP in animals and humans (Formelli et al. 1987 and 1989, Schaffer et al. 1993). A single dose of 4-HPR reduced the secretion of retinol from rat liver into plasma after the initial uptake of chylomicron vitamin A (Smith et al. 1992). Part of this effect is likely to be due to the ability of 4-HPR to fit into the retinol-binding cavity of RBP and, apparently due to the larger size of 4-HPR compared with retinol, to interfere with the protein-protein association of RBP and transthyretin (Berni et al. 1993). Because unbound RBP (21 kDa) is susceptible to loss from plasma through renal filtration or uptake into the extravascular space, disruption induced by 4-HPR may account for the observation that retinol enters the extravascular space more readily in 4-HPR–treated rats (Ritter et al. 1995).

Several metabolites of 4-HPR have been described in vivo or in vitro including N-(4-methoxyphenyl)-retinamide (Formelli et al. 1987, Hultin et al. 1990) and esters of 4-HPR (Hultin et al. 1990). It has been postulated that retinoic acid also is a metabolite of 4-HPR and may be the active form in vivo. Based on the ability of microsomes prepared from liver and other tissues to convert 4-HPR to retinoic acid in vitro (Shih et al. 1988), it seems plausible that 4-HPR is slowly hydrolyzed to retinoic acid in vivo. However, Shih et al. (1988) did not find retinoic acid as a liver metabolite.
of 4-HPR in vivo, possibly due to the rapid turnover of retinoic acid. The effects of 4-HPR that we observed on LRAT activity could have been mediated by 4-HPR per se, a metabolite, or through an indirect effect of 4-HPR on the metabolism of biological retinoids. We reported previously that LRAT activity is negligible in livers of vitamin A–deficient rats but is induced rapidly following treatment with vitamin A (Randolph and Ross 1991) or retinoic acid (Matsuura and Ross 1993). In the present studies, a single dose of ≥0.1 mg 4-HPR effectively induced liver LRAT activity (Table 2). This dose is comparable to or less than the daily doses that have been effective in studies of cancer chemoprevention (Hill and Grubbs 1992, Moon et al. 1979), or in producing a rapid reduction in plasma retinol and RBP concentrations (Formelli et al. 1987). Quantitatively similar effects were determined following an intraperitoneal dose of 2–20 μg of retinoic acid (Matsuura and Ross 1993). The effect of 4-HPR was also rapid (Fig. 1) and required in vivo administration (Table 1). The increase in LRAT activity induced by 4-HPR reached a maximum after 24 h and then began to decline. This decay suggests that the continuous presence of retinoid, which is known to turn over rapidly, is required for the maintenance of liver LRAT activity, and may also suggest that LRAT itself has a relatively short half-life. Pharmacokinetic studies in mice showed a decline to 10% of maximum values for plasma and liver 4-HPR in 24 h [Hultin et al. 1988]. The maintenance of tissue levels of 4-HPR (or retinoic acid) in a range adequate to maintain LRAT activity may require the frequent [e.g., daily] administration of this retinoid. The complete inhibition of the in vivo response to 4-HPR by pretreatment with actinomycin D (Fig. 2) suggests that the induction of liver LRAT activity by 4-HPR, as by retinoic acid (Matsuura and Ross 1993), requires new RNA and protein synthesis. To date, the LRAT enzyme has not been purified or its gene cloned; thus, antibodies and molecular reagents are not yet available to further test the hypothesis that liver LRAT mRNA or protein are increased by retinoid treatment.

It was previously reported that the addition of 4-HPR to microsomes in vitro competitively inhibited the esterification of retinol by ARAT (Ball et al. 1985), while other investigators reported a competitive inhibition between 4-HPR and CRBP-bound retinol for LRAT in intestinal microsomes or after detergent solubilization of LRAT [Dew et al. 1993]. However, neither group of investigators treated animals with 4-HPR in vivo. The significant correlation (r = 0.802, P < 0.0002) between induced liver LRAT activity assayed with CRBP-retinol under optimal in vitro enzyme assay conditions and the esterification in vivo of a pulse dose of 3H-retinol supports the conclusion that the in vitro results reflect the liver’s physiological capacity to esterify retinol. In vitamin A–sufficient rats fed 4-HPR–supplemented diet for 30 d, the activity of hepatic LRAT assayed with CRBP-retinol also increased. However, after 30 d of 4-HPR treatment, the liver’s total retinol mass was not different from that of untreated rats (Table 3). This result is similar to that of Moon et al. (1979) but, interestingly, differs from that of Adams et al. (1995) who determined a 1.5-fold increase in liver vitamin A content in rats treated chronically with 4-HPR while being fed a marginal level of vitamin A (about 0.75 μg/g diet) to maintain a low mass of liver vitamin A (averaging 1501 and 644 nmol retinol/total liver in 4-HPR–treated and control rats, respectively). The diet consumed by the normal rats in our study contained 4 μg retinol/g diet, and the liver vitamin A concentration of these rats was much higher (800–900 nmol total retinol/g for livers weighing ~8 g). It is possible that hepatic retinol catabolism as well as retinol esterification was increased during 4-HPR treatment under our dietary conditions.

It has previously been established that the jejunum is the major site of intestinal absorption for vitamin A [Rigtrup et al. 1994]. Treatment of normal rats for 30 d with dietary 4-HPR had no effect on LRAT activity in the duodenum plus jejunum (Table 3). Consistent with this, Allen et al. (1994) found no effect of chronic treatment with 4-HPR on intestinal retinol absorption measured as vitamin A uptake into the chyle of thoracic lymph duct–cannulated rats. However, Dew et al. (1993) reported that 4-HPR added to intestine microsomes or solubilized LRAT enzyme in vitro competitively inhibited LRAT activity. The in vitro effects of 4-HPR on LRAT activity may reflect physical effects

### Table 3

<table>
<thead>
<tr>
<th>Dietary treatment</th>
<th>Plasma retinol</th>
<th>Liver retinol</th>
<th>Liver LRAT activity</th>
<th>Duodenum + jejunum</th>
<th>Ileum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μmol/L</td>
<td>nmol/g</td>
<td></td>
<td>pmol retinyl ester/(min·mg homogenate protein)</td>
<td></td>
</tr>
<tr>
<td>Vehicle control</td>
<td>0.6 ± 0.2</td>
<td>908 ± 135</td>
<td>11.2 ± 1.3</td>
<td>4.0 ± 1.7</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>4-HPR</td>
<td>0.1 ± 0.2*</td>
<td>803 ± 103</td>
<td>19.3 ± 1.5*</td>
<td>2.6 ± 1.1</td>
<td>0.8 ± 0.4b</td>
</tr>
</tbody>
</table>

1 Values are means ± SD for 6 rats per group. *Significantly different from control, P < 0.0001; bsignificantly different from control, P < 0.002.
2 Female rats were fed purified diet containing vehicle or 2 mmol of 4-HPR/kg diet for 30 d.
3 As reported in Zhao et al. (1994) in which the same rats were studied for natural killer cell activity.

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**Effects of 4-hydroxyphenyl retinamide (4-HPR) on plasma and liver retinol and the activity of lecithin:retinol acyltransferase (LRAT) measured in homogenates of liver and small intestine from vitamin A–sufficient rats**
induced by intercalation of retinoid into membranes. In our experiments, 4-HPR did not induce LRAT in vitro in vitamin A–deficient microsomes, consistent with a need for new enzyme synthesis, and had only a small effect on LRAT activity when added directly to normal rat liver microsomes.

In summary, treatment in vivo with 4-HPR resulted in a significant increase in hepatic LRAT activity in both vitamin A–deficient and –sufficient rats. This change could influence the availability of hepatic retinol for secretion to plasma or for conversion to retinoid products via other metabolic pathways. Further studies are required to determine whether other aspects of hepatic retinol metabolism, such as its oxidation to retinoic acid, are also altered during acute or chronic treatment with 4-HPR.

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LITERATURE CITED


Smith, J. E., Lawless, D. C., Green, M. H. & Moon, R. C. (1992) Secretion of vitamin A and retinol-binding protein into plasma during steatorrhea is depressed in rats by N-(4-hydroxyphenyl)-all-trans-retinamide and N-(4-hydroxyphenyl)-all-trans-retinamide, the major metabolite of N-(4-hydroxyphenyl)-all-trans-retinamide in mice. Drug Metab. Dispos. 16: 783–788.